

What is claimed is:

1. A proteolytically active NP which has at least about 80% amino acid sequence homology with reference sequence thrombin and has a ratio of protein  
5 C activating activity to fibrinogen clotting activity that is less than about half of, or greater than about twice that of reference sequence thrombin, provided, however, that the novel polypeptide is not a naturally-occurring thrombin, thrombin K52E, thrombin R70E, thrombin R68E, thrombin K154A, thrombin K252E, thrombin K174E, thrombin R180E, thrombin D99A, thrombin D99N,  
10 thrombin E202Q, thrombin E25K, thrombin R245E, thrombin S205A, R197E, D199E, thrombin N151D, K154E, thrombin desP48P49W50, thrombin desE146T147W148, thrombin in which at least one amino acid residue within the thrombin activation site has been substituted or deleted, or thrombin in which loop F19-E25 is replaced by the equivalent loop from tissue plasminogen  
15 activator.
2. An isolated proteolytically active NP which has at least about 80% amino acid sequence homology with reference sequence thrombin and has a ratio of protein C activating activity to fibrinogen clotting activity that is less than about  
20 half of, or greater than about twice that of reference sequence thrombin, provided, however, that the novel polypeptide is not thrombin K52E, thrombin R70E, thrombin R68E, thrombin K154A, thrombin K252E, thrombin K174E, thrombin R180E, thrombin D99A, thrombin D99N, thrombin E202Q, thrombin E25K, thrombin R245E, thrombin S205A, R197E, D199E, thrombin N151D,  
25 K154E, thrombin desP48P49QW50; thrombin desE146T147W148, thrombin in which at least one amino acid residue within the thrombin amino activation site has been substituted or deleted, or thrombin in which loop F19-E25 is replaced by the equivalent loop from tissue plasminogen activator.
- 30 3. The NP of claim 1 wherein the NP is selected from a thrombin wherein one or more of residues W50, K52, D58, K65, H66, Y71, N74, K106, K107, S176, T177, W227, D193, K196, E202, E229, R233, D232, D234, K236, Y237 or F239 have been substituted, deleted or another residue inserted adjacent thereto.

4. The NP of claim 3 wherein W50 is deleted or another residue selected from the following group has been substituted therefor or inserted immediately adjacent thereto: G, A, V, I, L, S, T, D, N, E, Q, C, K, M, F, Y, P, R, and H.
- 5 5. The NP of claim 3 wherein K52, K65, K106, K107 or K196 is deleted or another residue selected from the following group has been substituted therefor or inserted immediately adjacent thereto: G, A, V, I, L, S, T, D, N, E, Q, C, M, F, Y, P, W, R, and H.
- 10 6. The NP of claim 3 wherein D58, D193 or D234 is deleted or another residue selected from the following group has been substituted therefor or inserted immediately adjacent thereto: G, A, V, I, L, S, T, N, E, Q, C, K, M, F, Y, P, W, R, and H.
- 15 7. The NP of claim 3 wherein residues W50 and K52, E229 and W50, R233 and W50, R233 and K52, or R233 and E229 are substituted or deleted.
8. The NP of claim 3 wherein H66 is deleted or another residue selected from the following group has been substituted therefor or inserted immediately  
20 adjacent thereto: G, A, V, I, L, S, T, D, N, E, Q, C, K, M, F, Y, P, W and R.
9. The NP of claim 3 wherein Y71 is deleted or another residue selected from the following group has been substituted therefor or inserted immediately adjacent thereto: G, A, V, I, L, S, T, D, N, E, Q, C, K, M, F, P, W, R, and H.  
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10. The NP of claim 3 wherein N74 is deleted or another residue selected from the following group has been substituted therefor or inserted immediately adjacent thereto: G, A, V, I, L, S, T, D, E, Q, C, K, M, F, Y, P, W, R, and H.
- 30 11. The NP of claim 3 wherein E202 or E229 is deleted or another residue selected from the following group has been substituted therefor or inserted immediately adjacent thereto: G, A, V, I, L, S, T, D, N, Q, C, K, M, F, Y, P, W, R, and H.

12. The NP of claim 3 wherein the substitution, deletion or insertion is made only in the A or B chain.

13. The NP of claim 3 wherein R233 is deleted or another residue selected  
5 from the following group has been substituted therefor or inserted immediately adjacent thereto: G, A, V, I, L, S, T, D, N, E, Q, C, K, M, F, Y, P, W, and H.

14. The NP of claim 3 wherein residues D193, K196 and K52 are substituted or deleted.

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15. The NP of claim 3 wherein the residue is substituted and the substitution is with alanine.

16. The NP of claim 3 wherein 2 or 3 of the residues are substituted.

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17. The NP of claim 3 which comprises B chain free of A chain.

18. The NP of claim 3 which comprises A and B chain.

20 19. The NP of claim 1 which possesses greater than about 2 times the residual proteolytic activity of reference thrombin when measured by hydrolysis of S-2238 in the presence of heparin-dependent AT-III inhibition.

20. The NP of claim 3 wherein a heparin binding site residue is substituted.

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21. The NP of claim 20 wherein the residue is R89, R180, R245, K248 or K252.

22. The NP of claim 3 which is K52A, R233A NP, E229D NP, E229F NP, E229S NP, E229W NP, E229Y NP, R233N NP, R233D NP, R233F NP, W50C NP, K52C NP, W50E NP or W50K NP.

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23. The NP of claim 1 wherein the thrombin has been substituted at residues K21, Q24, R70, R98 or K77, one or more of such residues have been deleted or another residue has been inserted immediately adjacent to one or more of such  
35 residues.

24. The NP of claim 3 wherein the residue is E229 or R233.

25. The NP of claim 1 wherein the residue is within about 10 Angstroms of  
5 the C $\alpha$  of E229 or R233.

26. A nucleic acid encoding the NP of claim 1.

27. A replicable vector comprising the nucleic acid of claim 26.  
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28. A recombinant cell comprising the vector of claim 27.

29. A method comprising culturing the cell of claim 28 and recovering the NP  
from the cell culture.  
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30. The method of claim 29 wherein the NP is expressed in the cell culture as  
a soluble polypeptide.

31. The method of claim 29 wherein the NP is expressed in the cell culture as  
20 the B chain alone.

32. The method of claim 29 wherein the nucleic acid encodes A and B  
sequences each one of which is independently ligated to nucleic acid encoding a  
signal sequence and the nucleic acid is coexpressed in the same host cell.  
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33. A method for the treatment of thrombotic diseases or conditions  
comprising administering a therapeutically effective amount of a proteolytically  
active NP which is at least about 80% homologous by amino acid sequence with  
reference sequence thrombin and has a ratio of protein C activating activity to  
30 fibrinogen clotting activity that is greater than about twice that of reference  
sequence thrombin.

34. The method of claim 33 wherein the NP possesses greater than about  
twice the residual proteolytic activity of reference thrombin when measured by  
35 hydrolysis of S-2238 in the presence of heparin-dependent AT-III inhibition.

35. The method of claim 33 wherein the thrombotic disease or condition is cardiac bypass surgery.

5 36. The method of claim 33 wherein the NP is devoid of detectable fibrinogen clotting activity.

37. The method of claim 36 wherein the NP retains at least about 5% of wild-type thrombin protein C activating activity.

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38. The method of claim 37 wherein the NP is K52A, R233A NP, E229D NP, E229F NP, E229S NP, E229W NP, E229Y NP, R233D NP, R233N NP, R233F NP, W50C NP, K52C NP, W50E NP, or W50K NP.

15 39. An NP which is at least about 80% homologous by amino acid sequence with reference sequence thrombin in which at least one amino acid residue has been substituted, deleted or inserted immediately adjacent to thrombin residues R20, K21, S22 Q24, E25, D35, W250, D51, K52, N53, F54, T55, N57, D58, K65, H66, R68, T69, R70, Y71, R73, N74, K77, E82, E83, R89, R93, R94, R98, K106, K107,  
20 D113, C119, D122, R123, E124, S128, Q131, K145, E146, T147, W148, T149, N151, K154, S158, E169, K174, D175, S176, T177, R178, I179, R180, D183, D193, K196, D199, A200, C201, E202, N216, N217, W227, G228, E229, G230, C231, D232, R233, D234, G235, K236, Y237, G238, F239, R245, K248, Q251, R245, K248, W249, Q251, K252, D255, or Q256, provided, however, that the NP is not thrombin K52E,  
25 thrombin R70E, thrombin R68E, thrombin K154A, thrombin K252E, thrombin K174E, thrombin R180E, thrombin D99A, thrombin D99N, thrombin E202Q, thrombin E25K, thrombin R245E, thrombin S205A, R197E, D199E, thrombin N151D, K154E, thrombin desP48P49QW50, thrombin desE146T147W148, thrombin in which at least one amino acid residue within the thrombin amino  
30 activation site has been inserted, substituted or deleted, or thrombin in which loop F19-E25 is replaced by the equivalent loop from tissue plasminogen activator.